doi:10.1017/S1431927616003767
Microsc. Microanal. 22 (Suppl 3), 2016

Imaging a 1 mm^3 Volume of Rat Cortex Using a MultiBeam SEM

R. Schalek[1], D. Lee[2] N. Kasthuri[3], A. Peleg[1,4], T. Jones[1,4], V. Kaynig[1,4], D. Haehn[4], H. Pfister[1,4], D. Cox[1,2,4] and J.W. Lichtman[1,2].

[1.] Center for Brain Science, Harvard University, Cambridge, MA USA.
[2.] Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA USA.
[3.] Department of Nanoscience, Argonne National Labs, Lemont, IL, USA.
[4.] J. A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA USA.

The rodent brain is organized with length scales spanning centimeters to nanometers --6 orders of magnitude [1]. At the centimeter scale, the brain consist of lobes of cortex, the cerebellum, the brainstem and the spinal cord. The millimeter scale have neurons arranged in columns, layers, or otherwise clustered. Recent technological imaging advances allow the generation of neuronal datasets spanning the spatial range from nanometers to 100s of microns [2,3]. Collecting a 1 mm^3 volume dataset of brain tissue at 4 nm x-y resolution using the fastest signal-beam SEM would require ~6 years. To move to the next length and volume scale of neuronal circuits requires several technological advances. The multibeam scanning electron microscope (mSEM) represents a transformative imaging technology that enables neuroscientists to tackle millimeter scale cortical circuit problems. In this work we describe a workflow from tissue harvest to imaging that will generate a 2 petabyte dataset (> 300,000,000 images) of rat visual cortex imaged at a 4nm x 4nm x-y (Nyquist sampling of membranes) and 30nm section thickness in less than 6 months.

The anatomical pipeline starts with perfusion and dissection of rat visual cortex followed by vibratoming of a ~3mm x 3mm x 1 mm slice. An EM block is prepared using a reduced–osmium thiocarbohydrazide osmium staining protocol followed by epon embedding of a 1 mm^3 piece of cortex. As seen in Figure 1a, a custom RMC ATUMtome with a 1 mm sectioning-arm travel is used to collect 33,333, 30nm serial sections on a continuous length of carbon-coated Kapton tape. After collecting the sections (Figure 1b), segments of tape are adhered to ~170 silicon wafers (Figure 1c) creating an ultrathin-section library.

A project of this scale requires substantial workflow automation and performance monitoring to minimize human labor, increase throughput, and guarantee high-quality data. As an example of the dataset scale consider the data plotted in Figure 2a where the number of terabytes per day generated, and the total number of imaging days required to acquire a 1mm^3 volume are plotted for the current imaging speed of 200 Mpixels/s and for the expected data acquisition speeds of 250 and 300 Mpixels/s. The image rates of 250 and 300 Mpixels/s should be realizable with a new stage damping mechanism, an image tile pitch increase from 12 um to 15 um, and by replacing mSEM autofocus operations with optically generated focus maps. These improvements increase the number of files per hour from 88,362 to ~132,000 (Figure 2b) and further increase the requirements for automation, and quality monitoring.

Some illustrative examples of the unique mSEM image configuration is shown in Figure 3. In Figure 3a, a single multifield of view (mFoV) is comprised of 61 image tiles with a 12 um pitch with a horizontal field width of 100 um, while Figure 3 b shows a mosaic of 7 mFoVs covering an area almost 90,000 um^2. Finally, the 4 nm pixel and ~2.5 nm beam resolution cortex ultrastructure and annotated axon and dendrites are illustrated in Figure 3c.

References:

[1] JW Lichtman and W Denk, Science **4** (2011) p. 618.
[2] N Kasthuri, KJ Hayworth, et al., Cell **162** (2015), p. 648.
[3] J Morgan, D Berger, et al., Cell (2016) in press.
[4] The authors acknowledge funding from IARPA Contract # D16PC00002.

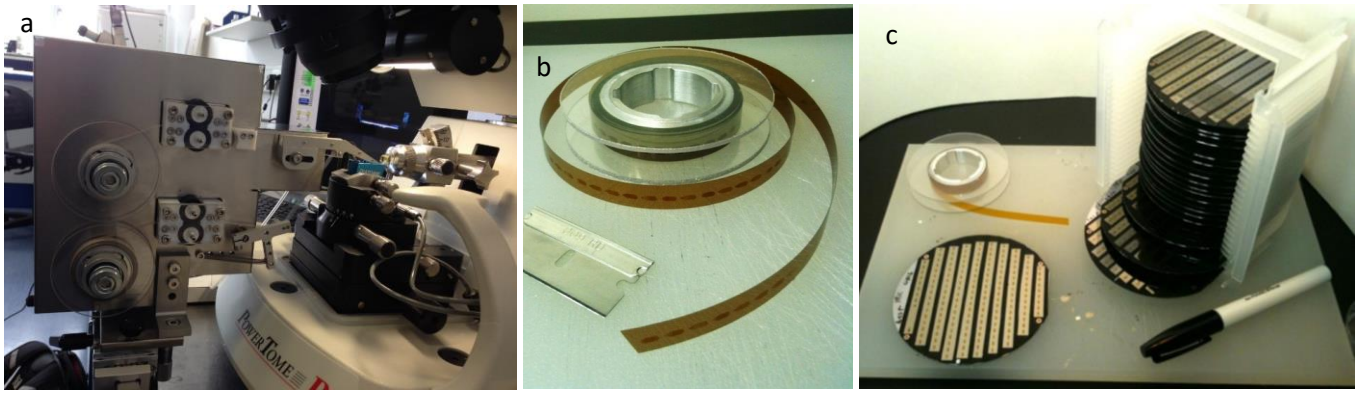

Figure 1. (a) ATUMtome used to collect 30 nm thick serial sections. (b) a typical reel of tape with sections (dark rectangles) collected on tape. (c) library of wafers containing thousands of serial sections.

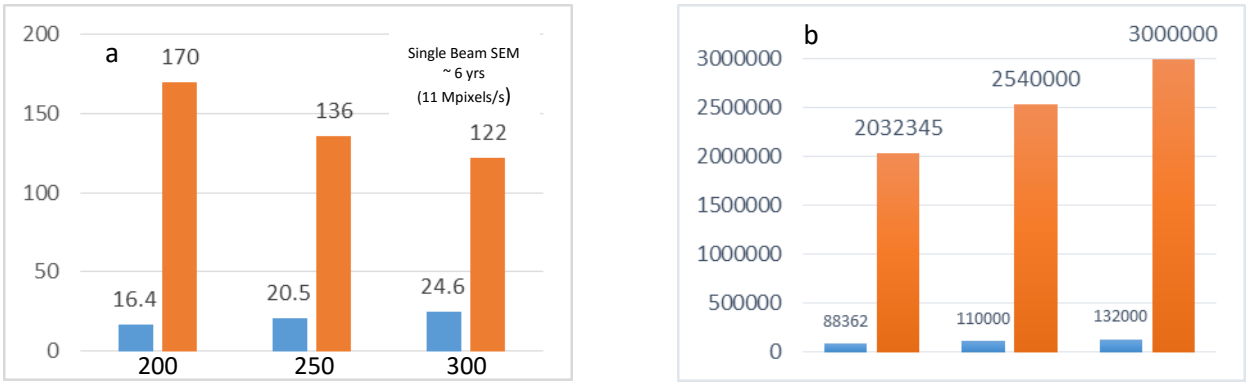

Figure 2. (a) the number of terabytes per day at 200, 250 and 300 Mpixels/s (blue), the number of imaging days (orange). (b) approximate number of files generated per hour (blue) and per day (orange).

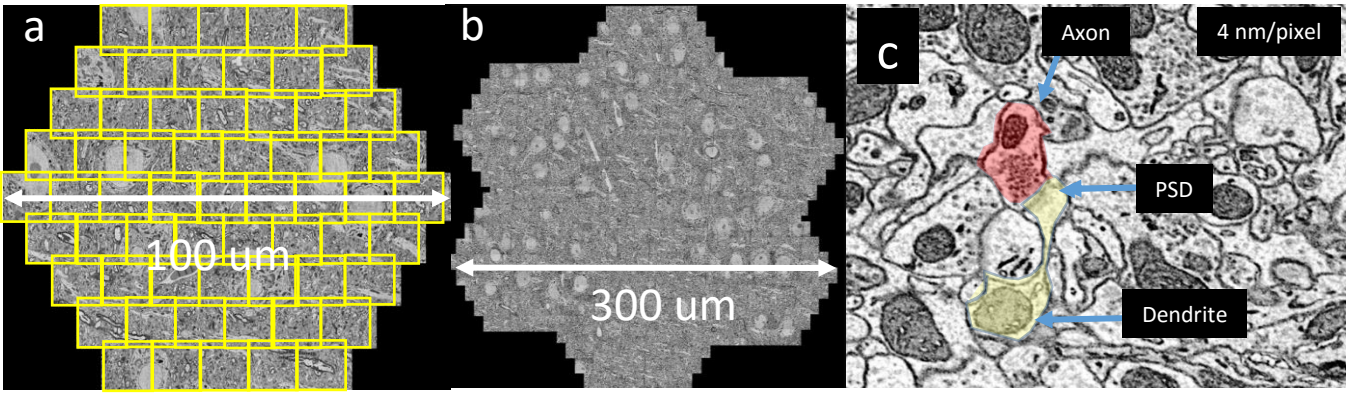

Figure 3. (a) typical mFov with 61 images collected simultaneously (2.2 s). (b) a stitched image of 7 mFoVs. (c) an image of neuropil illustrating a synaptic connection.